Histone H2A/H2B Dimer Human, Recombinant

Source: Purified H2A and H2B (NEB #M2502 and NEB #M2505) were denatured, refolded and purified by gel filtration.

Note: To use as a substrate in an enzyme modification assay or other salt sensitive protocol, use 1 to 2 µl of the dimer in a minimum 20 µl reaction so that the salt concentration in the reaction ≤ 200 mM.

Protein Concentration: 20 µM (0.55 mg/ml) calculated using the molar extinction coefficient of Histone Dimer (11,920) and its absorbance at 280 nm (4,5).

Quality Control Assays:

SDS-PAGE: 2.0, 4.0, 8.0 µg of Histone H2A/H2B Dimer Human, Recombinant were loaded on a 10–20% Tris-Glycine SDS-PAGE gel and stained with Coomassie Blue. The calculated molecular weights of the two subunits found in this protein complex are 13,990.28 Da for Histone H2A and 13,788.97 Da for Histone H2B. Their apparent molecular weight on 10–20% Tris-Glycine SDS-PAGE gel are ~14 kDa and ~14.5 kDa.

Protease Assay: After incubation of 10 µg (~360 pmol) of Histone H2A/H2B Dimer Human, Recombinant with a standard mixture of proteins for 4 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Exonuclease Assay: Incubation of a 50 µl reaction containing 10 µg (~360 pmol) of Histone H3.1/H4 Tetramer Human, Recombinant with 1 µg of a mixture of single and double-stranded [H] E. coli DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Assay: Incubation of a 50 µl reaction containing 10 µg (~360 pmol) of Histone H2A/H2B Dimer Human, Recombinant with 1 µg of xK174 RF I (supercoiled) plasmid DNA for 4 hours at 37°C resulted in < 5.0% conversion to RF II form (nicked circle) as determined by agarose gel electrophoresis.

References:
3. Mersha, F., unpublished observations

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SDS-PAGE analysis of Histone H2A/H2B Dimer Human, Recombinant.

Lanes 1 & 2: Histone H2A (NEB #M2502S)
Lanes 3 & 4: Histone H2B (NEB #M2505S)
Lanes 5–7: 2, 4, 8 µg Histone H2A/H2B Dimer

Supplied in: 20 mM Tris-HCl, pH 8.0 @ 25°C, 2 M NaCl, 1 mM EDTA, 1 mM DTT. Store at –20°C.

(Because of high salt, the solution does not always remain frozen.)

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Tetramer Human, Recombinant with 1 µg of a mixture of single and double-stranded [H] E. coli DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Assay: Incubation of a 50 µl reaction containing 10 µg (~360 pmol) of Histone H2A/H2B Dimer Human, Recombinant with 1 µg of xK174 RF I (supercoiled) plasmid DNA for 4 hours at 37°C resulted in < 5.0% conversion to RF II form (nicked circle) as determined by agarose gel electrophoresis.

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